REMARKS

Claims 56-70 and 76-86 were pending in the subject application. By this Amendment, applicants have canceled claims 76-86 without prejudice or disclaimer, amended claims 56 and 63, and added new claims 87-89. Accordingly, upon entry of this Amendment, claims 56-70 and 87-89 will be pending and under examination.

The amendment places the claims in condition for allowance or in better form for appeal. Applicants maintain that the amendments to the claims do not raise an issue of new matter. Support for the amendments to claim 56 can be found in the previous versions of the claim and *inter alia* in the specification at least on page 11, line 19 through page 12, line 12, page 18, lines 9-10, and in Figure 2. Support for the amendment to claim 63 can be found in the previous version of the claim. New dependent claims 87-89 recite features from claim 56. Support for new claims 87-89 can be found in claim 56. Accordingly, entry of the Amendment is respectfully requested.

Rejections under 35 U.S.C. §112, Second Paragraph

A) Claims 56-62 are rejected as indefinite over the phrase "*nucleotides* set forth in." Claim 56 has hereinabove been amended to replace this phrase with "nucleic acid sequence ... set forth in." Applicants maintain that the amendment renders the claims definite and accordingly respectfully request reconsideration and withdrawal of this ground of rejection.

B) Claims 63-70 are rejected as indefinite over the recitation of "a protein comprising twelve transmembrane domains and amino acid residues Asp 16, Glu 79 and Arg 208." Claim 63 has hereinabove been amended to delete this text thereby rendering this rejection moot.

C) Claims 80-86 are rejected as indefinite over the recitation of "the nucleotide sequence." Claims 80-86 have hereinabove been canceled thereby rendering

this rejection moot.

Rejections under 35 U.S.C. §112, First Paragraph

A) Claims 63-70 are rejected under the written description and enablement requirements of 35 U.S.C. §112, first paragraph, for the full scope of the claims.

Applicants have hereinabove amended claim 63 to delete the phrase on which the Examiner based the rejections, i.e. "a protein comprising twelve transmembrane domains and amino acid residues Asp 16, Glu 79 and Arg 208." Applicants maintain that the teachings of the specification convey to the skilled artisan that at the time the application was filed the inventors had possession of the invention set forth in the amended claims and that the teachings of the specification enable the skilled artisan to practice the claimed invention without undue experimentation. Accordingly, reconsideration and withdrawal of these rejections are respectfully requested.

B) Claims 56-62 are rejected under 35 U.S.C. §112, first paragraph, as not enabled for the full scope of the claims.

Applicants have hereinabove amended claim 56 to recite "A method of determining whether a mammalian sodium/iodide symporter is expressed in a mammalian tissue, the method comprising contacting nucleic acid from the mammalian tissue with a probe and detecting whether the probe hybridizes to the nucleic acid, wherein detecting hybridization of the probe to the nucleic acid indicates that the mammalian sodium/iodide symporter is expressed in the mammalian tissue, wherein the probe comprises: (i) the nucleic acid sequence corresponding to the coding region set forth in SEQ ID NO:1 or its complement; (ii) a nucleic acid sequence that hybridizes to

the nucleic acid sequence corresponding to the coding region set forth in SEQ ID NO:1 or its complement to permit detection of a mammalian sodium/iodide symporter expressed in a mammalian tissue by hybridization; or (iii) a nucleic acid sequence that hybridizes to a portion of the nucleic acid sequence corresponding to the coding region set forth in SEQ ID NO: 1 or its complement to permit detection of a mammalian sodium/iodide symporter expressed in a mammalian tissue by hybridization."

Applicants maintain that the teachings of the specification enable the skilled artisan to practice the claimed invention without undue experimentation. The skilled artisan could readily detect the expression of a mammalian sodium/iodide symporter using a probe that comprises the nucleic acid sequence corresponding to the coding region set forth in SEQ ID NO:1 or its complement. The coding region of SEQ ID NO:1 is set forth in Figure 2. Both strands of the sodium/iodide symporter cDNA were sequenced by the inventors (page 18, lines 9-10 of the specification). The complement of SEQ ID NO:1 is readily apparent from SEQ ID NO:1. Similarly, the skilled artisan could readily use a probe comprising a nucleic acid sequence that hybridizes to the nucleic acid sequence corresponding to the coding region set forth in SEQ ID NO:1 or its complement to detect expression of a mammalian sodium/iodide symporter. Finally, applicants maintain that the skilled artisan could, without undue experimentation, use a nucleic acid sequence that hybridizes to a portion of the nucleic acid sequence corresponding to the coding region set forth in SEQ ID NO: 1 or its complement to detect a mammalian sodium/iodide symporter expressed in a mammalian tissue by hybridization.

There was a high level of skill in the art of using detection probes as of the February 1, 1996 filing date of the parent of the subject application. For example, the prior art taught the relation between the length of a probe and the specificity of hybridization. See "The Effects of Length and Degeneracy of the Oligonucleotide on the

Specificity of Hybridization" in Sambrook, J., et al., Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989, pp 11.7-11.8, a copy of which is attached hereto. Sambrook et al. state on page 11.7 that "In the case of mammalian genomes, ... an oligonucleotide of 14-15 nucleotides would therefore be expected to be represented only once in the genome." On page 11.4, in a section entitled "Single Oligonucleotides of Defined Sequence" (copy attached), Sambrook et al. state:

> Probes consisting of a single nucleotide of defined sequence usually correspond to part of the sequence of a previously cloned segment of DNA. In general, such oligonucleotides match with their target sequence perfectly, or nearly perfectly, and they are sufficiently long (19-40 nucleotides) to allow the use of hybridization conditions that can guarantee discrimination between the target sequence and other closely related sequences.

The above-mentioned references are attached as examples of the high level of skill in the art at the time of the subject application's priority date and should not be construed to limit the level of skill in the art at that time. The prior art examples are provided to show that the skilled artisan would be able to practice the claimed invention without undue experimentation. For example, the skilled artisan could use a probe 19-40 nucleotides in length, as taught by Sambrook et al. (1989), where the nucleotides correspond to a portion of the nucleic acid sequence corresponding to the coding region set forth in SEQ ID NO: 1, to hybridize to a portion of the complement of the nucleic acid sequence corresponding to the coding region set forth in SEQ ID NO: 1 and to thus detect a mammalian sodium/iodide symporter expressed in a mammalian tissue by hybridization.

Accordingly, in view of the amendments and remarks made hereinabove, reconsideration and withdrawal of this ground of rejection are respectfully requested.

C) Claims 80-86 are rejected under the enablement requirement of 35 U.S.C. §112, first paragraph. Claims 80-86 have hereinabove been canceled thereby rendering this rejection moot.

CONCLUSION

In view of the amendments and remarks made hereinabove, applicants respectfully request that the Examiner reconsider and withdraw the rejections set forth in the March 22, 2004 Final Office Action and earnestly solicit allowance of the claims under examination, namely claims 56-70 and 87-89. If there are any minor matters that would prevent allowance of the subject application, the Patent Office is requested to contact the undersigned attorney.

No fee is deemed necessary in connection with the filing of this response. However, if any fee is required to preserve the pending of the subject application, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 01-1785. Overpayments may also be credited to Deposit Account No. 01-1785.

Respectfully submitted,

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Dated: New York, New York
May 20, 2004

By _____

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2

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

J. Sambrook
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

E.F. Fritsch
GENETICS INSTITUTE

T. Maniatis
HARVARD UNIVERSITY

Cold Spring Harbor Laboratory Press
1989

SINGLE OLIGONUCLEOTIDES OF DEFINED SEQUENCE

Probes consisting of a single oligonucleotide of defined sequence usually correspond to part of the sequence of a previously cloned segment of DNA. In general, such oligonucleotides match with their target sequence perfectly, or nearly perfectly, and they are sufficiently long (19–40 nucleotides) to allow the use of hybridization conditions that can guarantee discrimination between the target sequence and other closely related sequences. Single oligonucleotides of defined sequence are used:

- To screen cDNA or genomic DNA libraries, or subclones derived from them, for additional clones containing segments of DNA that have been isolated previously and sequenced
- In Southern, northern, or dot-blot hybridization to identify or detect the sequences of specific genes
- In Southern or dot-blot hybridization of genomic DNA to detect specific mutations in genes of known sequence
- To detect specific mutations generated by site-directed mutagenesis of cloned genes
- To map the 5′ termini of mRNA molecules by primer extension

Oligonucleotide probes used for these purposes are usually labeled by phosphorylation of 5′ termini with [γ-^{32}P]ATP (see pages 11.31–11.32) or, more rarely, by synthesis of a radioactive complementary strand with the Klenow fragment of *E. coli* DNA polymerase I (see pages 11.40–11.44). Because the base composition and size of each oligonucleotide probe are known precisely, conditions can be defined that allow hybridization of the oligonucleotide to its desired target and little or no hybridization to closely related sequences that are not perfectly matched (see below). Obviously, such probes are limited to applications where the sequence of the target DNA or RNA is already known (see, e.g., Montgomery et al. 1978); they are of no use when screening libraries to isolate clones of unknown sequence.

The Effects of Length and Degeneracy of the Oligonucleotide on the Specificity of Hybridization

The number of independent perfect matches (P_0) expected for an oligonucleotide of length L in a genome of complexity C can be calculated from the equation:

$$P_0 = (1/4)^L \cdot 2C$$

The complexity, which is measured in nucleotide pairs, is the sum of the nucleotide pairs that occur in unique (single-copy) sequences and those that occur in one copy of each repeated sequence. Thus, nucleotide pairs belonging to repetitive elements, which may occur many times in a genome, are counted only once. Only approximate values of C are known for genomes that have not been sequenced completely. The following estimates are taken from Laird (1971).

Genome	Size[a]	Complexity[a]
Escherichia coli	4.0×10^6	$\sim 4.0 \times 10^6$
Yeast	1.4×10^7	$\sim 1.4 \times 10^7$
Drosophila	1.1×10^8	$\sim 1.05 \times 10^8$
Mammals	2.9×10^9	$\sim 1.8 \times 10^9$

[a]Numbers are given in nucleotide pairs.

The term $2C$, rather than C, is used in the equation because the sequence of each strand of DNA is different and has the potential to hybridize to the probe. As the length of an oligonucleotide increases, the chance that it will find an exact match in the genome of interest decreases. When $4^L = 2C$, the sequence of the oligonucleotide would be expected to occur only once in the genome. In the case of mammalian genomes, $2C \cong 3.6 \times 10^9$, and an oligonucleotide of 14–15 nucleotides would therefore be expected to be represented only once in the genome. However, the distribution of nucleotides in the coding sequences of mammalian genomes is nonrandom (Lathe 1985), and it is therefore advisable to use longer oligonucleotides in order to increase the specificity of hybridization. For example, when the oligonucleotide is 16 nucleotides long, there is only one chance in ten that a typical mammalian cDNA library ($C \cong 10^7$ nucleotides) will fortuitously contain a sequence that exactly matches the sequence of the oligonucleotide. Thus, any clones that hybridize strongly to the probe are likely to be derived from the gene of interest. Bear in mind that when a cDNA library is screened with an oligonucleotide probe, there is no relationship between the observed number of positive clones and their frequency predicted by statistics. For example, if by chance the oligonucleotide should match a sequence that is abundantly represented in mRNA, the number of clones that hybridize to the probe will be much larger than theory predicts.

The effect of hybridization between imperfectly matched sequences cannot be easily quantitated, since different types of mismatch (mispairing between single bases, loopouts on either strand, multiple mismatches closely or distantly spaced) have different effects on the stability of double-stranded DNA. For example, a single mismatch in the center of a short oligonucleotide (14–16 nucleotides in length) will eliminate any possibility of hybridization

under any practicable conditions. However, a mismatch at the end of such an oligonucleotide, or in the center of a longer oligonucleotide, may have no detectable effect (Ikuta et al. 1987).

Assuming that all mismatches at all positions are equivalent, the number of clones (P_K) that will hybridize detectably to an oligonucleotide of length L with K mismatches is given by the equation:

$$P_K = (1/4)^{L-K} \cdot (3/4)^K \cdot 2C \cdot L!/[K! \cdot (L-K)!]$$

For the simplest case (that of a single mismatch, where $K = 1$):

$$P_1 = (1/4)^{L-1} \cdot (3/4) \cdot 2C \cdot L$$

Compare this with the number of perfect matches:

$$P_0 = (1/4)^L \cdot 2C$$

The ratio $P_1/P_0 = 3L$. Thus, if the hybridization conditions allow detection of singly mismatched sequences, a 24-mer will hybridize to 72-fold more mismatched clones than perfectly matched clones.

When the oligonucleotide is close to the critical length (which for screening of a typical mammalian cDNA library is 17–18 nucleotides [Lathe 1985]), it is worthwhile increasing its length in order to maximize its specificity. The concomitant increase in mismatches that occurs as the degeneracy of the pool increases is not a significant problem when dealing with such small oligonucleotides, since mispaired sequences are unlikely to be stable under practicable conditions of hybridization. Since the decrease in the stability of a hybrid is approximately 1–1.5°C for every percentage of mismatch, the melting temperature of a hybrid 20 nucleotides in length would be reduced 5–7.5°C by internal mismatches. This is sufficient leeway to allow easy discrimination between perfectly matched and internally mismatched hybrids formed by short (<20 nucleotides) oligonucleotides. However, as the length of the oligonucleotide increases beyond 20 nucleotides, the effect of mismatches on the melting temperature of the hybrid becomes progressively less significant, and it becomes difficult to establish hybridization conditions that will discriminate between perfectly matched and internally mismatched hybrids. When the oligonucleotide is more than 20 nucleotides long, many mismatches are likely to be stable under the conventional conditions used for hybridization. This can result in the isolation of an unacceptably high number of candidate clones, many of which will turn out not to contain the gene of interest. In summary, oligonucleotides in degenerate pools should be long enough to hybridize specifically to the gene of interest and short enough not to form internally mismatched hybrids that can be detected by hybridization. For example, screening of mammalian cDNA or genomic DNA libraries should be carried out with degenerate pools of oligonucleotides 17–20 nucleotides in length. When longer probes are used, it is usually necessary to take steps to limit the degeneracy of the pool as much as possible (see pages 11.11–11.19).